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(54) **Method and system for processing scan-data from a confocal mircoscope**

(57) The invention discloses a method for processing scan signals from a confocal microscope and a confocal microscope with a system for processing scan-signals. The confocal microscope comprises an illumination source (2) and a scanning device (6) with a scanning mirror system (7). A control and processing unit (16) is provided, which uses a plurality of programmable devices for processing the digital signals in real-time. The control and processing unit (16) has at least three input ports (16_1, 16_2, 16_3) and one output port (16_4). A first detector (12) generates analog signals from light reflected from a specimen (10) within the microscope and a second detector (14) generates analog signals proportional to the intensity of the light from the illumination source (2). In addition a position signal of the scanning laser beam is provided to the control and processing unit (16). Analog digital converters (30_1, 30_2, 30_3) are located prior to the input ports (16_1, 16_2, 16_3) of the control and processing unit (16) for providing digital signals, generated from the analog signals from the first and second detector and the position signal of the scanning device.

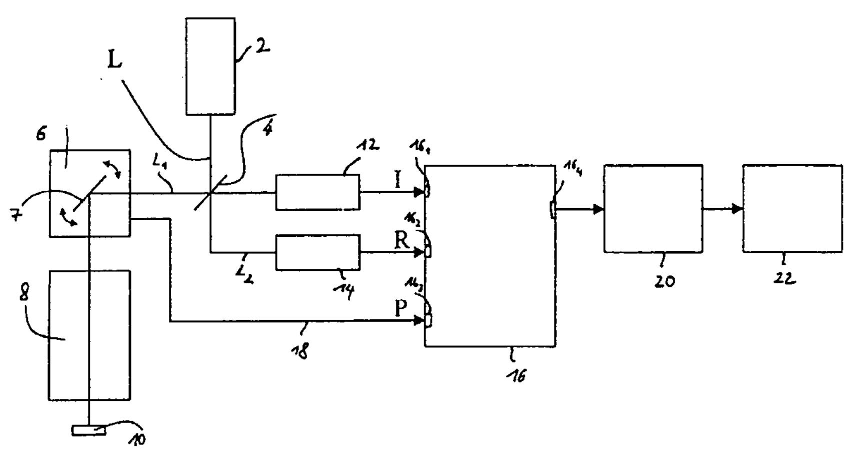

Fig. 1

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Description

[0001] This invention relates to a method and a system for processing scandata form a confocal microscope. More specifically the invention relates to a method an system which enables real-time processing of the scanned data.

[0002] In confocal microscopy a specimen is scanned with a focussed laser beam. The focus of the light beam is moved in a section plane of a specimen by tilting two scan mirrors around their axes. The axes of the scan mirrors are perpendicular to each other. For example one scan mirror diverts the laser light in the x-direction and the other scan mirror diverts the laser light in the y-direction. The power of the reflected or the fluorescent light is measured for each scanning point. Each measured value, with respect to power, relates to a x, y and z-position of the specimen. The user achieves a three-dimensional image of the specimen.

[0003] An example for a confocal microscope is discolsed in the U.S. Patent 5,804,813 to Wang Jyh Pyng et al., entitled "Differential confocal microscopy". The present invention uses a He-Ne laser as the light source, and the focusing device is a microscope objective lens. The reflected light from the almost completely reflected to an optical detector , which can use photodiodes, avalanche photodiodes, photo multipliers, charge coupled devices (CCDs), or fluorescent screens. The signal is detected by the optical detector and then amplified by a signal amplifier. The amplified signal is recorded by an analog-to-digital converter and then stored by a computer. The computer generates the three-dimensional image by using the intensity of the signal combined with the respective coordinate of the specimen. Before the measurement it is necessary to use the same sample to calibrate the relationship between the variation of signal intensity and the height of the sample.

[0004] The ideal scan pattern of the light beam on the surface of a specimen is a meander (to scan one line in the x-direction with a constant y-position, then stop the scan in the x-direction and move the y-position to the next scan line; then this line is scanned in the opposite x-direction and so on). In the real world the meander form does not exist for high scan rates. This is due to the inertia of the galvanometric devices and the scanning mirrors. The real path of the scanning beam has, at scan rates (> 100Hz), a sinusodial form which requires a correction to the ideal path. Several error types may occur, for example, a higher velocity of the scanning point at the turning point of the real path and differing shape of the scan path for different scan directions. In addition to that, the different run and process times for the intensity and the position signal respectively have to be considered.

[0005] As disclosed by the prior art above registration and processing of the position signal is normally done with an analog circuit, with computers or digital signal processors (DSP). There are certain disadvantages of signal processing with analog circuits. For example, signal processing can only be carried out with a correction function implemented in the analog circuit. Changes of the correction mechanism, for example change of the scan rate, requires a lot of effort. Moreover the accuracy of analog circuits with respect to mathematical operations reaches a limit at high scan rates. Pixel rates greater than one MHz at a 12 bit accuracy can be achieved only with enormous effort.

[0006] Signal processing with a computer is or DSP is possible at low scan rates. Furthermore the signal processing is also flexible since the algorithms for processing can be changed without a major effort. At high scan rates computers or DSP fail due to the lack of real-time ability.

[0007] It is an object of the present invention to provide a system for processing scan-signals from a confocal microscope in digital form and real-time. Moreover the accuracy of the measurement should be governed solely by the accuracy detectors the accuracy of the digital analog converters. Furthermore, the processing of the scan-signals should be possible at high scanning rates.

[0008] This object is achieved by a system which comprises:

at least three different analog signal are generated the by confocal microscope;

at least three analog digital converters each receiving a different analog signal and produces respective digital signals at an early stage; and
a control and processing unit which uses a plurality of programmable devices for processing the digital signals in real-time.

[0009] It is also an object of the present invention to provide a confocal microscope with a system for processing scan-signals which allows the processing of the scanned signals at high scanning rates and real time. Additionally, there should be a high flexibility which allows for example an easy alteration of the used algorithms.

[0010] This object is accomplished by confocal microscope which comprises: a illumination source,

a scanning device with a scanning mirror system
a control and processing unit, which uses a plurality of programmable devices for processing the digital signals in real-time, having at least three input ports and one output port,
a first detector generates analog signals from light reflected from a specimen;
a second detector generates analog signals proportional to the intensity of the light from the illumination source;
an electrical connection providing an analog position signal, generated by the scanning device, to the control and processing unit; and

a first, second and third analog digital converter are located prior to the input ports of the control and processing unit for providing digital signals, generated from the analog signals from the first and second detector and the scanning device.

[0011] Another object of the present invention is to provide a method for processing scan-signals from a confocal microscope. The method should enable processing of the analog scan-signals at high scanning rates. Furthermore the method should have a real time ability so that a flexible an loss free data collection is possible.

[0012] This object is achieved by a method which comprises the following steps:

generating at least three different analog signals by the confocal microscope wherein a first signal represents light reflected from a specimen, a second signal represents an illumination reference and a third signal represents a position on the specimen;

converting each analog signal with a separate analog digital converters into respective digital signals;

providing the digital signals to a control and processing unit which uses at least one of programmable devices for processing the digital signals in real-time;

buffering the digital position signals in a line-buffer of the control and processing unit;

correcting the first digital signal for intensity fluctuations of the second digital signal for generating a corrected digital detection signal; and

combining a specific digital position signal with a specific digital detection signal.

[0013] An advantage of the inventive method and the system is that the position signal of the focused scanning beam and signals from the detectors (scanning beam and reference beam) are digitized at a very early stage. Processing of the data is done mainly in digital form with the use of a programmable control and processing unit which is realized by a programmable digital circuit (for example FPGA, Field Programmable Gate Array). Correction parameters can be used online, which would make downstream image processing much easier or obsolete. The accuracy of the device is governed solely by the accuracy of the detectors and the analog digital converters. Analog digital converters with a big processing band width are available at reasonable costs. The position correction function can be programmed with respect to the requirements resulting from the measurement conditions. An alteration of the position correction function can be done online. The dynamic pixel accumulation records several measurement values during the scan of an image pixel and accumulates the data in a suitable buffer. The dynamic pixel accumulation allows a wide dynamic range form a few Hz to some MHz (right now the upper limit is 250 MHz) this make high scanning rates possible.

[0014] Pixel accumulation calls for a simple analog circuit without giving up the high resolution and the wide dynamic range. Despite the fact, that the analog digital converters enable only sampling of intensity signals from a narrow band of signal heights, the scanning several times and the pixel accumulation result in a theoretical unlimited bit resolution.

[0015] With the pixel accumulation the electronic processing is adjusted in time to the scan-velocity. Thereby, during a slow scan, the registered intensity signals are accumulated until the scanning beam has reached the next pixel element of the object. The pixel rate can be adjusted to the internal processing rate by pixel accumulation.

[0016] The advantages of pixel accumulation are analog to line accumulation (multiple sequential scan of one scan line and addition of the intensity data in a suitable buffer arrangement). Moreover, differences in the line-scan in one direction from the line scan in the other direction are averaged by line accumulation. In case of resonant galcanometers for the x-scan, a pixel accumulation is not possible so that line accumulation has to be used only.

[0017] A further advantage of the system is its real time ability, which repeats predetermined process steps with an accuracy up to nanoseconds. The inventive system fulfills the requirements of flexibility and real time ability. There is no need for buffer memories, since a computer controlled data recording is not used. The real time ability of the inventive system guaranties a loss free and flexible data collection with the highest, at present possible, scan rates.

[0018] Computers can also be programmed, but they are controlled by interrupts so that a running process have to be ended before further data processing can go on. This is, with respect to the present invention, also not considered a real-time ability. The present invention has the ability of a high flexibility with respect to data process changes and real-time processing of data.

[0019] The subject matter of the invention is described with reference to the embodiments shown in the drawings.

Fig. 1;shows a schematic illustration of a confocal microscope and a system for achieving three dimensional specimen images;

Fig. 2 is an embodiment of a process unit;

Fig. 3 shows an ideal path of the focus of the scanning beam; and

Fig. 4 shows an actual path of the focus of the scanning beam.

[0020] Fig. 1 shows a schematic illustration of a confocal microscope and a system for achieving three dimensional specimen images. An illumination system 2 generates a light beam L. A beam splitter 4 divides the

incident light beam L into a first path L_1 and into a second path L_2. With the first path L_1 the light from the illumination system is directed to a scanning device 6. The scanning device 6 comprises a scanning mirror system 7 which is moveable such, that the light of the first path L_1 is scanned across a specimen 10. Before the light of the first path L_1 reaches the specimen 10 the light passes an optical system 8. The light reflected from the specimen travels on12 the first path L_1 back to the beam splitter 4. After the beam splitter 4 a first detector 12 is positioned, which receives the light reflected from the specimen 10. The first detector 12 converts the received light into an first electrical signal I which is proportional to the intensity of the received light received form the specimen. A second detector 14 receives directly via the second path L_2 the light from the illumination system 2. As the first detector 12 the second detector 14 converts the received light into an second electrical signal R which is proportional to the intensity of the received light, which is a illumination reference. A control and processing unit 16 is provided which has a first second and third input port 16_1, 16_2 and 16_3. The first electrical signal I is fed to the first input port 16_1 and the second electrical signal R is fed to the second input port 16_2. The third input port 16_3 receives via an electrical connection 18 a position signal P which is generated in the scanning device 6. The disclosed embodiment describes three different analog signals I, R and P which are fed to the control and processing unit 16. With the use of multiplexers (not shown) more than three different signal can be provided to and processed with the control and processing unit 16.

[0021] The control and processing unit 16 converts the incoming analog signals (first electrical signal I, second electrical signal R and position signal P) which may be distorted and disrupted in into corrected digital signals. Via an exit port the digital signals are sent to a computer 20 which may carry out some image processing. A conventional display 22 is used to show the image of the specimen 10 to an user of the system.

[0022] An embodiment of the control and processing unit 16 is shown in Fig. 2. The control and processing unit 16 is realized with a plurality of FPGA-units (Field Programmable Gate Array). The first analog electrical signal I enters a first analog-digital converter 30_1,the second analog electrical signal R enters a second analog-digital converter 30_2 and the analog position signal P enters a third analog-digital converter 30_3. From the first analog-digital converter 30_1 and from the second analog-digital converter 30_2 the digitized signal are fed to a first and a second delay-modules 32 and 34 respectively which are used to carry out run-time differences. The use of the first and second delay-module 32 an 34 is important since the first and second electric signal I and R have a processing time which is different to the position signal P. This means that the reaction time of the scanning mirror system 7 is delayed due to its inertia. The delag modules eliminate the possible error due to its own processing time and reaction time differences. The output of the first and second delay module 32 and 34 is fed to the first and second input port 16_1 and 16_2 respectively of the control and processing unit 16. Here the digital signal from the first delay-module 32 is sent to a first adder 40 followed by a first buffer 42. There is a loop 70 between the first buffer42 and the first adder 40 to enable a pixel accumulation. From the first buffer 42 the data are sent to a first look-up-table 43 which carries out a log-conversion of the data. From the second delay-module 34 the digital signal is sent to a second adder 50 followed by a second buffer 52. There is a loop 80 between second the buffer 52 and the second adder 50 to enable a pixel accumulation. From the second buffer 52 the data are sent to a second look-up-table 53 which carries out a log-conversion of the data. From the third analog-digital converter 303 the positional data are sent to a third adder 60 followed by a third buffer 62. There is a loop 90 between the third buffer 62 and the third adder 60 to enable a pixel accumulation. From the third buffer 62 the data are sent to an address look-up-table 63 which carries out correction of the position data with respect to the scan along the x-direction. In order to obtain the correct digital data for the position of the scan beam there may be different look-up tables, one for each direction of the scan in the x-direction.

[0023] From the first look-up-table 43 and the second look-up-table 53 the data are fed to a calculation device 44. The calculation device 44 subtracts the data representative of the first electrical signal I from the data representative of the second electrical signal R. This enables a correction of the short term intensity fluctuations of the laser output signal. The data from the calculation device 44 are fed to a fourth look-up-table 45, which carries out a conversion of the data with respect to the basis e. The result of this conversion is that the data are available as the quotient of data representing the first electrical signal I and data representing the second electrical signal R. The data from the forth look-up table 45 are fed to a forth adder and from there to a line buffer 47 which correlates a specific digital position signal with a specific digital detection signal. There is also a loop 48 between the line buffer 47 and the forth adder 46 to enable a line accumulation. The position data are from the address look-up-table 63 are sent also to the line buffer 47. From the line buffer 47 the corrected digital signals are sent to via the exit port 164 of the processing unit 16 to a data input port 100 of the computer 20.

[0024] In most cases the devices used to tilt the scanning mirror system 7 are galvanometers. In general, both resonant and non-resonant galvanometers are used. The resonant galvanometers are very fast (approx. several kHz), but they are not as exact as the non-resonant galvanometers, which are much slower. Fig. 3 shows and ideal path 110 of the focus of the scanning beam across specimen area of interest 112. The dashed line represents the ideal path 110. The ideal path 110 comprises a plurality of linear movements 114 of the fo-

cused beam in a direction x of the specimen area 112. The linear movements 114 are separated by a constant line feed 116 which has a direction y, perpendicular to the linear movements 114. In other words the ideal path 110 of the focus of the scanning beam would be a back and forth pattern of parallel lines in the direction x which are separated by the constant line feed 116 in the direction y. The line feed 116 is done by tilting the y-mirror (not shown), thereby moving the focus of the scanning beam to the next line. This next line is scanned with the x-mirror (not shown) followed by another line feed 116 and so on until the whole specimen area 112 is scanned.

[0025] The ideal path 110 as shown in Fig. 3 can not be achieved, because of the inertia of the mirrors and the and the moving galvanometer elements. Actually, a real path 120 across the specimen area of interest 112 is shown in Fig. 4. The form of the real path is sinusoidal. According to the differences between the ideal path 110 and the actual path 120 a correction of this difference is necessary. Furthermore, it has to be considered that the velocity of the focus of the scanning beam in the direction x increases at turning points 122 compared to linear regions 124 of the sinusoidal curve. In order to run a microscope at considerable high scanning rates, a real time processing unit (field programmable gate array, FPGA) is used to correct and process the detector and position signals (for example, to provide a correction for the differences as shown in Fig. 3 and Fig.4).

[0026] The FPGA provides also a correction in the case of an asymmetric scan path (the scan path in the x-direction differs from the scan path in the opposite direction). This requires a different correction for the scan path in the x-direction and another correction for the scan path in the opposite direction. In other words, the position signal has to be corrected with respect to the different paths of the scanning beam across the object.

[0027] The invention has been described in detail with particular reference to certain preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention.

PARTS LIST

[0028]

2	illumination system
4	beam splitter.
6	scanning device
7	scanning mirror system
8	optical system
10	specimen
12	first detector
14	second detector
16	control and processing unit
16_1	first input port
16_2	second input port
16_3	third input port
16_4	exit port
18	electrical connection
20	computer
22	display
30_1	first analog-digital converter
30_2	second analog-digital converter
30_3	third analog-digital converter
32	first delay-module
34	second delay-module
40	first adder
42	first buffer
43	first look-up-table
44	calculation device
45	forth look-up-table
46	forth adder
47	line buffer
48	loop1
50	second adder
52	second butter
53	second look-up-table
60	third adder
62	third buffer
63	address look-up-table
70	loop
80	loop
90	loop
100	computer
110	ideal path
112	specimen area of interest
114	linear movement
116	line feed
120	real path
122	turning point
124	linear region
I	first electrical signal
R	second electrical signal
P	position signal
L	light beam
L_1	first path
L_2	second path
X	scan direction
Y	scan direction

Claims

1. A system for processing scan-signals from a confocal microscope, the system characterized in that:

   at least three different analog signal (I, R, P) are generated the by confocal microscope;
   at least three analog digital converters (30_1, 30_2, 30_3) are provided for receiving a different analog signal and producing respective digital signals at an early stage; and
   a control and processing unit (16) uses at least one programmable device for processing the digital signals in real-time.

2. The system as recited in claim 1 wherein the programmable device is a field programmable gate array.

3. The system as recited in claim 1 wherein the three different analog signals (I, R, P) are generated by the confocal microscope, the three different signals comprise a first electrical signal (30_1) which is proportional to the intensity of the light received form the a specimen (10), a second electrical signal (30_2) which is an illumination reference and a third electrical signal (30_3) which is a position signal of an actual position of a scanning light beam on the specimen (10).

4. The system as recited in claim 3 wherein the control and processing unit (16) comprises a address look up table (63) to provide a correction for the position signal (P).

5. The system as recited in claim 4 wherein the position signal (P) generated from a scan in the x-direction is different from a scan in the opposite direction x-direction with respect to time, and the correction of the position signal (P) is chosen with respect to the scan direction.

6. The system as recited in claim 1 wherein the control and processing unit (16) comprises a calculation device (44) to correct short term intensity fluctuations, and a line buffer (47) to correlate a specific digital position signal with a specific digital detection signal.

7. The system as recited in claims 1 to 6 is incorporated in a confocal microscope, wherein the confocal microscope comprising:

   an illumination source (2);
   a scanning device (6) with a scanning mirror system (7);
   a first detector (12) generates analog signals (I) from light reflected from the specimen (10);
   a second detector (14) generates analog signals (R) proportional to the intensity of the light from the illumination source (2); and
   an electrical connection (18) providing an analog position signal (P) generated by the scanning device, to the control and processing unit (16).

8. A method for processing scan-signals from a confocal microscope, is characterized by the following steps:

   generating at least three different analog signals (I, P, R) by the confocal microscope wherein a first signal (I) represents light reflected from a specimen (10), a second signal (P) represents an illumination reference and a third signal (R) represents a position on the specimen (10);
   converting each analog signal with a separate analog digital converter (30_1, 30_2, 30_3) into respective digital signals;
   providing the digital signals to a control and processing unit (16) which uses a plurality of programmable devices for processing the digital signals in real-time;
   buffering the digital position signals in a line-buffer of the control and processing unit (16);
   correcting the first digital signal (I) for intensity fluctuations of the second digital signal (R) for generating a corrected digital detection signal; and
   combining a specific digital position signal with a specific digital detection signal.

9. The method as recited in claim 8 wherein the control and processing unit (16) comprises programmable devices which are field programmable gate arrays.

10. The method as recited in claim 8 comprises an additional step of providing a correction for the position signal (P) with the control and processing unit (16), wherein the correction is implemented in a address look up table (63).

11. The method as recited in claim 10 wherein the correction of the position signal (P) is done by providing a correction for the scan in the x-direction and a different correction for the scan in the opposite x-direction.

12. The method as recited in claim 8 wherein a first look-up table (43) carries out a log conversion of the first electrical signal (I).

13. The method as recited in claim 8 wherein a second look-up table (53) carries out a log conversion of the second electrical signal (R).

14. The method as recited in claim 8 comprises the steps of: providing the combined digital position signal and the digital detection signal to a computer (20); and

   displaying and image of the scanned specimen area.

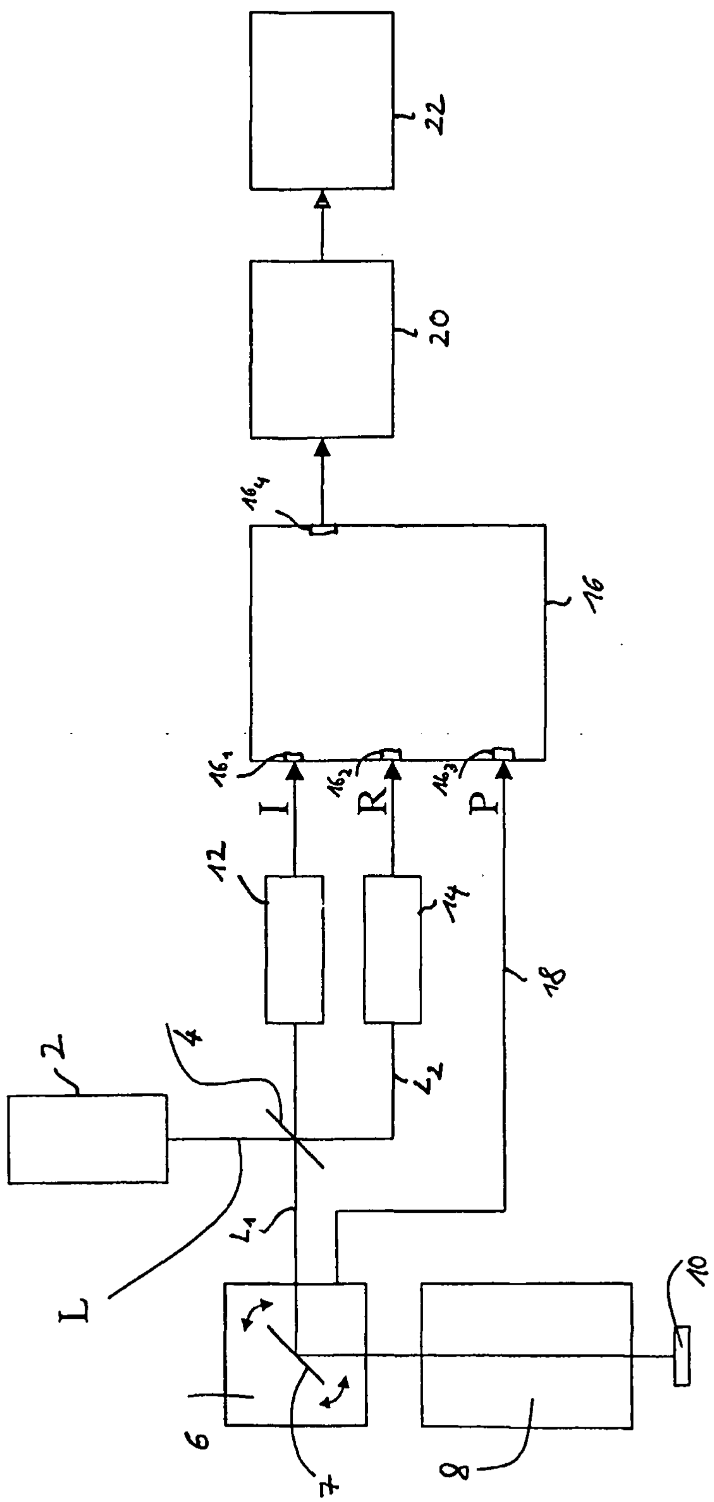

Fig. 1

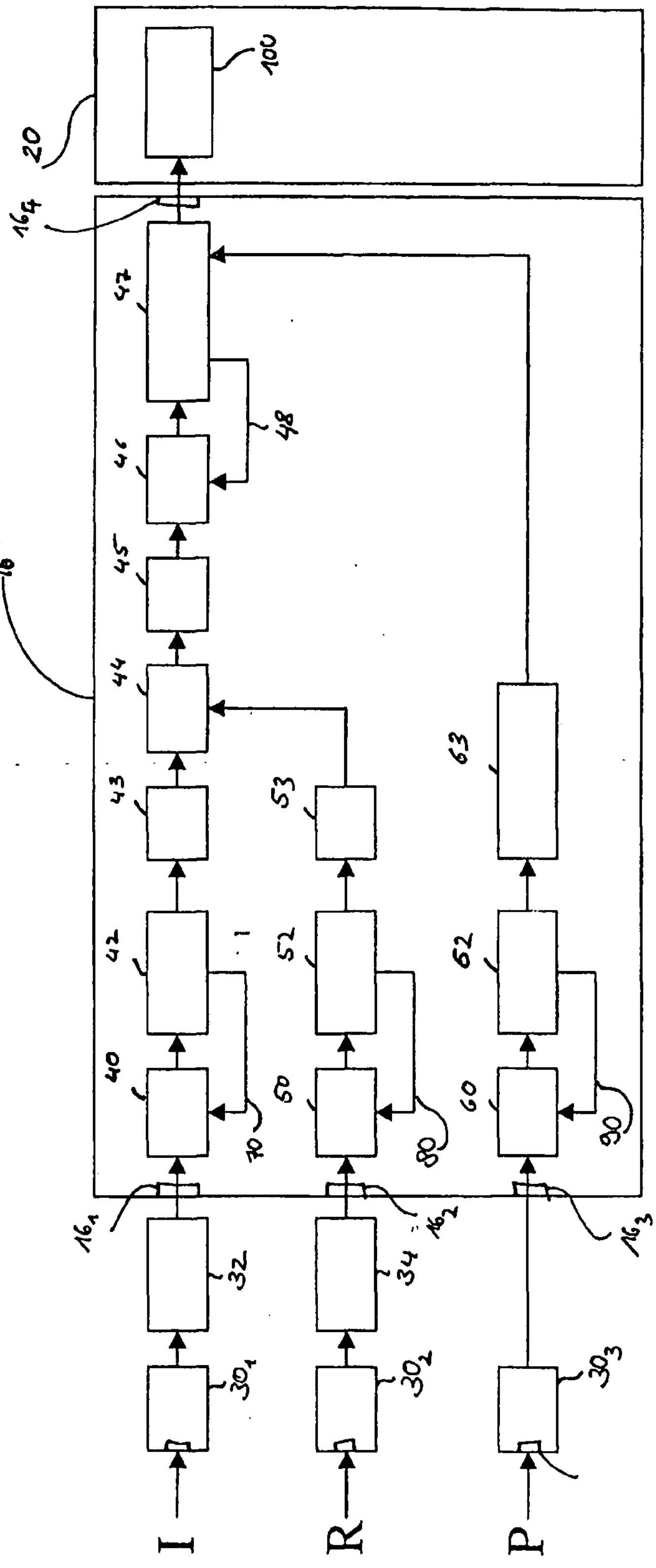

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Fig. 2

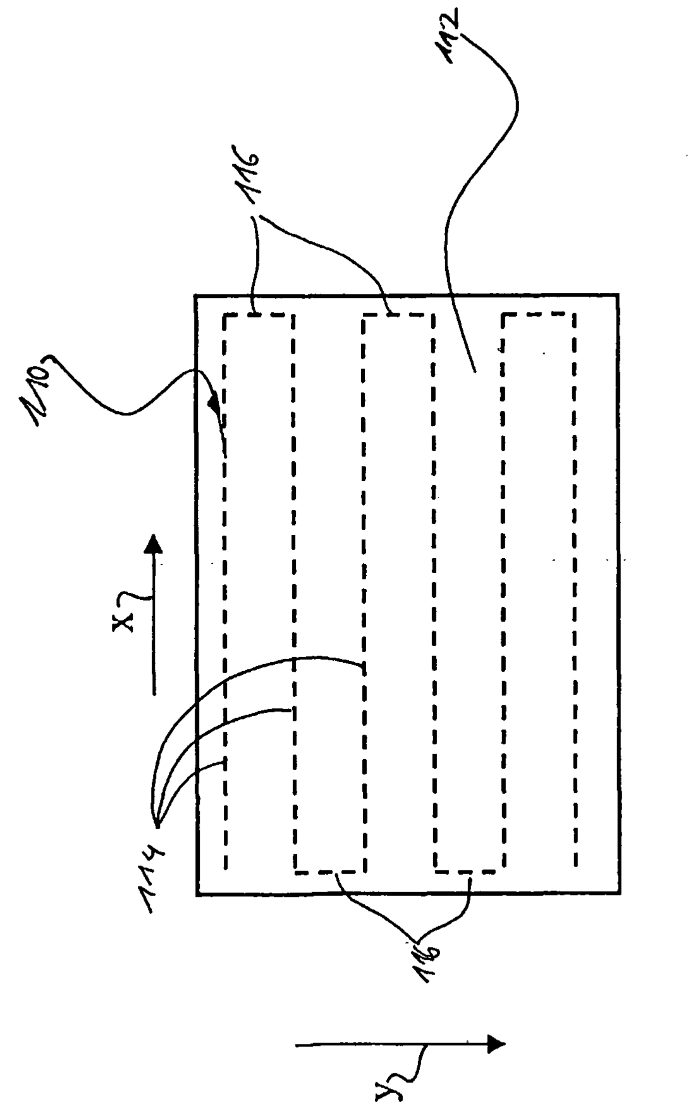

Fig. 3

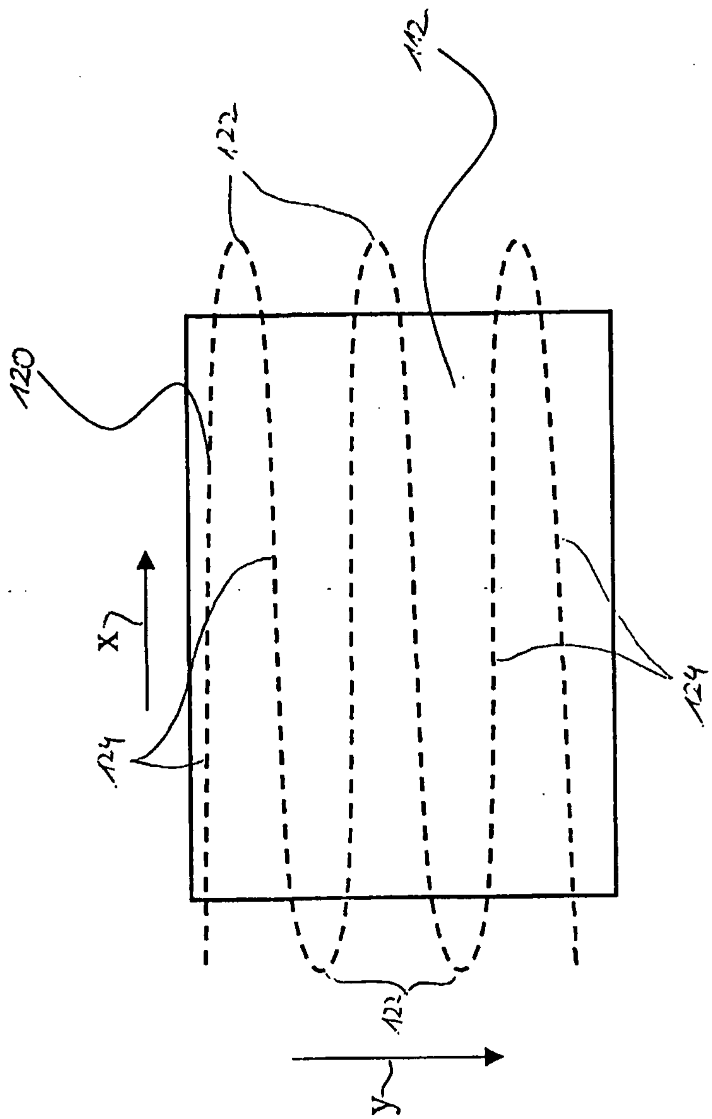

Fig. 4